

Sortase A

Week 1

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Testing conjugation between spider silk and a peptide

2

1 Testing conjugation between spider silk and a peptide

Responsible

Aman Mebrahtu and Oscar Frisell

Protocols used

Sortase A Conjugation
SDS-PAGE

Modifications and comments to protocols

The reaction proceeded for 30 minutes at 37 °C instead of 3 hours at room temperature.

Experimental Data

Samples: 10 ml of 521 μM Z_{Q32C}-SR-H₆ was dissolved in 1x Sortase A binding buffer.

25 ml 10x Sortase A binding buffer

2 tubes with 50 μl of 1mM Sortase A in Sortase A storage buffer.

30 mg/ml Spider silk

Calculations

Total volume = 550 μl .

Amount of Z_{Q32C}-SR-H₆ = 230 μl $\Rightarrow (c_2 \cdot V_2)/(c_1) = (n_2)/(c_1) = V_1 = 120\text{nmol}/521\mu\text{l} = 230 \mu\text{l}$.

Amount of Sortase A Binding buffer = 55 μl \Rightarrow 1x in final volume, 10x in stock, $(1x/10x) \cdot \text{Total volume} = 0.1 \cdot 550 = 55\mu\text{l}$

Amount of Sortase A = 2.75 μl $\Rightarrow V_1 = (5\mu\text{M} \cdot 550\mu\text{l})/1\text{mM} = 2.75\mu\text{l}$

Amount of Spider silk with N-terminal = 231 μl $\Rightarrow M_w = 22.997\text{kDa}$

Concentration = 3 mg/ml = 130 nmol/ml

Volume = $(30\text{nmol}) / (130\text{nmol/ml}) = 231 \mu\text{l}$

Results and Conclusions

Table 1: Order of the samples in Figure 1.

Well	Sample	Date	Temperature for conjugation(°C)
1	Ladder		
2	Negative Control	28/6	37
3	Spidersilk + Z	28/6	37
4	Negative Control	7/7	37
5	Spidersilk + Z	7/7	37
6	Negative control	7/7	RT
7	Spidersilk + Z	7/7	RT
8	Ladder		

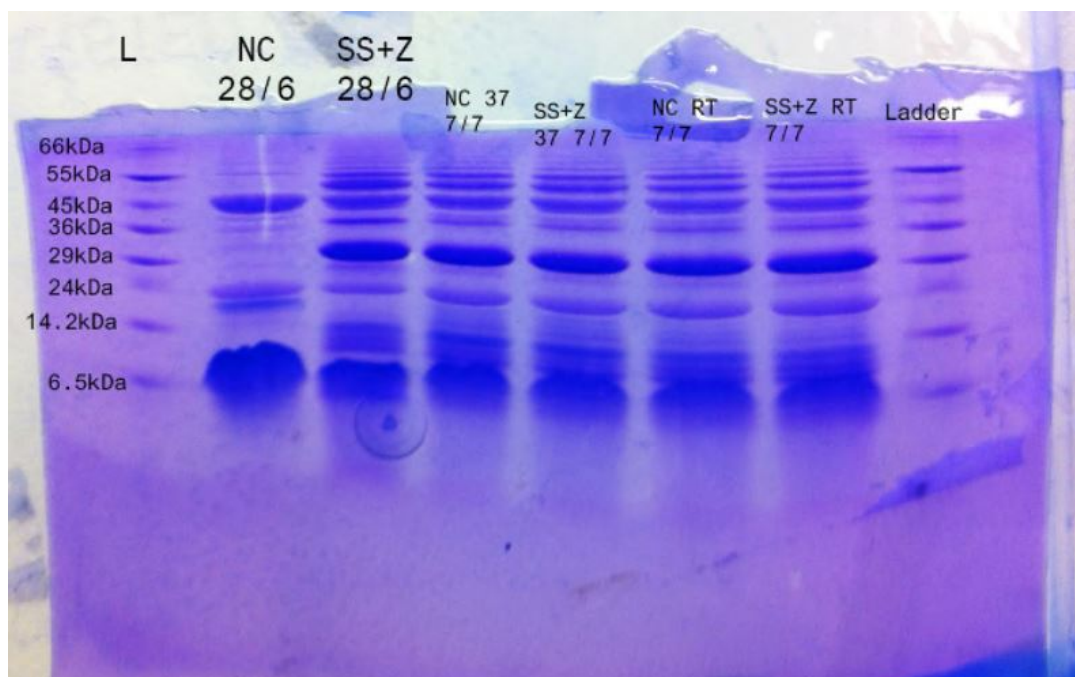


Figure 1: SDS-PAGE with samples described in Table 1.

Discussion and Troubleshooting

The sample in well 4-7 does not look good and has probably failed. The conjugation from 28/6 in well 3 looks as well a bit strange and have probably not worked either. The size should be SIZE and no clear band at that size can be seen.